# Summary Progress Report

CTR Grant #869

Progress Report #2
6/1/74 - 1/1/75

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Title of Grant: The Action of Nicotine on the Adrenal Gland

During the past year and a half our laboratory has been engaged in a comprehensive study of the stimulatory effects of nicotine on the cat adrenal gland. The major thrust of this investigation has been from two major perspectives: (a) to elucidate the role of cyclic AMP in the mechanism whereby nicotine, acetylcholine and other medullary secretogogues enhance catecholamine secretion and (b) to discern whether nicotine exerts a direct action on adrenocortical cells, and if so, by what mechanism. Since the details of this work are elaborated and conclusions amply documented in the accompanying reprint (Jaanus and Rubin, 1974) and preprint (Rubin and Warner, in press), only the highlights of these studies will be presented here.

(a) Medulla. Earlier work established that calcium is an absolute requirement for nicotine and acetylcholine-induced release of catecholamines by the cat adrenal medulla (Douglas and Rubin, 1961b). Since cyclic AMP has been implicated as a "second messenger" in many physiological and pharmacological responses (Robison et al., 1971), it is well understood that the elucidation of the nature of calcium action in nicotine-induced catecholamine release requires intimate knowledge of the role of cyclic AMP. Therefore, in collaboration with Dr. Siret Jaanus (Jaanus and Rubin, 1974), the effects of nicotine on cyclic AMP levels and catecholamine release were determined in cat adrenal glands perfused in situ with Locke's solution.

Initially, cyclic AMP analyses (method of Steiner et al., 1969) were carried out on the cat medulla and cortex, after separating these two organs. The mean basal levels in medulla and cortex were 30 ± 13 and 172 ± 6 pmoles/gland, respectively. Thus, establishing the presence of cyclic AMP in the medulla, experiments were carried out to discern if a quantitative and/or temporal correlation existed between medullary cyclic AMP levels and catecholamine secretion following stimulation by nicotine. Indeed, perfusion with nicotine or acetylcholine ($4 \times 10^{-5}$ g/ml) elevated adrenal cyclic AMP levels and enhanced its rate of release into the adrenal perfusate. However, the time course of the changes in tissue cyclic AMP during stimulation was out of phase with the time course of catecholamine release. Maximal increases in cyclic AMP were not manifest until after eight minutes of exposure to the secretogogue, whereas maximal rates of secretion occurred during the first minute. The lack of correlation between adrenal cyclic AMP levels and catecholamine release was also reflected in experiments with theophylline, an inhibitor of phosphodiesterase. Theophylline, in concentrations which augment adrenal cyclic AMP levels, failed to enhance basal catecholamine release and did not potentiate the secretory response to a submaximal concentration of nicotine.

These data are interpreted to mean that the elevation of secretion effected by nicotine is not directly monitored by adrenal cyclic AMP levels in a manner which resembles that of calcium. These findings do not mitigate the possibility, however, that one or more alternate mediators are interposed between the response triggered by nicotine and the release of hormone. Therefore, experiments are now in progress to determine whether the stimulatory action of nicotine on the adrenal medulla is better correlated with alterations in cyclic GMP and/or prostaglandin levels.

Cortex. Up to now, controversy has existed as to whether nicotine is able to elevate corticosteroid secretion by a direct action on adrenocortical cells (Kershbaum et al., 1968; Suzuki et al., 1973). Our recent work has clearly established (see preprint) that nicotine possesses the potentiality of directly stimulating isolated cat adrenocortical cells. The stimulant action manifests itself in micromolar concentrations, is dose-dependent and like the physiological stimulus, ACTH, depends upon the presence of calcium. Not only is nicotine able to stimulate cortical cells directly but it enhances the steroidogenic effect of ACTH in an additive, rather than synergistic, manner.

The ifurther observations that nicotine enhances the steroidogenic action of exogenous cyclic nucleotide and prostaglandin $E_2$, just as it enhances the action of ACTH, suggest that these proposed mediators of steroidogenesis play crucial roles in mediating the action of nicotine. However, support of such an hypothesis requires the measurement of changes in tissue levels of these putative mediators during stimulation by nicotine, and these experiments are now in progress. stimulation by nicotine. Indeed, perfusion with nicotine or acetylcholine ($4 \times 10^{-5}$ g/ml) elevated The role of calcium in the steroidogenic action of nicotine also cannot be defined at present. Nicotine-like acetylcholine stimulates the medulla to secrete by depolarizing the chromaffin cell membrane (Douglas et al., 1967), facilitating transmembrane calcium flux, which in turn triggers the release of preformed catecholamine stored in secretory granules (see Rubin, 1970). On the other hand, in the cortex there appears to be no correlation between steroidogenic activity and the depolarization of cortical cells (Jaanus et al., 1970), and stimulation by ACTH results from an intracellular translocation of calcium (Jaanus and Rubin, 1971). Since nicotine can traverse cell membranes and release calcium from cellular binding sites (Weiss, 1968) an intracellular mobilization of calcium may be responsible for nicotine-induced steroidogenesis. Experiments are now in progress to compare the effects of ACTH and nicotine on radiocalcium fluxes in the isolated cat adrenocortical preparation. Such studies will provide salient information as to whether the action of nicotine on the adrenal cortex is primarily associated with a transmembrane flux of extracellular calcium or a redistribution of intracellular calcium. triggered by nicotine and the release of hormone. Therefore, ex-

Although our studies have uncovered valuable information concerning the mode of action of nicotine on adrenal hormone release, we still have an incomplete picture of the processes involved in this action. Despite the recognized importance of nicotine, as a result of its widespread use, there have been surprisingly few experimental studies concerned with the mechanism by which this pharmacologic agent affects non-neuronal endocrine cells. There is therefore an obvious gap in our knowledge regarding cellular events occurring during nicotine-induced activation of the secretory process. Advances made in our knowledge of the intimate mechanism of nicotine action by a continuation of this study would have important implications and immense value in regard to understanding and treating the pathophysiological states related to the chronic use of tobacco.

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